Shared and Specific Susceptibility Loci for Schizophrenia and Bipolar Disorder

A Dense Genome Scan in Eastern Quebec Families

Maziade et al., Molecular Psychiatry (2005)

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Part I: Background & Study Pedigrees

Study Objective

Primary Goal: Identify shared and disorder-specific susceptibility loci for schizophrenia (SZ) and bipolar disorder (BP)

Key Questions:

- Do SZ and BP **share** common genetic susceptibility loci?
- Are there loci **specific** to each disorder?

Why study SZ and BP together?

- Family studies show co-aggregation of SZ and BP within pedigrees
- Previous linkage studies analyzed the disorders separately
- Separate analysis may cause diagnostic misclassification to break segregation patterns within pedigrees

Background: The Diseases

Bipolar I Disorder (BP):

- Mood disorder with manic and depressive episodes
- Lifetime prevalence of 0.6%; Bipolar spectrum disorders: 1-2%
- **Highly heritable (60-85%)**
- ~70% reported psychotic symptoms

Schizophrenia (SZ):

- Key features include hallucinations, delusions, disorganized speech, abnormal motor behavior, diminished emotional expression
- Lifetime prevalence in population is 0.3-0.7%
- **High heritability (70-80%)**
- **Schizoaffective Disorder:**
  - Schizophrenia with mood symptoms (mania and/or depression)
  - lifetime prevalence is ~0.3%

Complex Genetic Disorders

- Non-Mendelian inheritance with incomplete penetrance
  - Carrying a susceptibility allele does not guarantee disease expression
- Genetic heterogeneity
  - Similar clinical phenotypes may result from different susceptibility loci or mutations across families
- Phenocopies
  - Similar clinical phenotype induced by enviromental factors
  - Eg., prenatal or perinatal adversities including stress, infection, or other medical complications
- Other risk factors: neurodevelopmental factors, substance exposure, psychosocial stressors, etc.

Study Population: Eastern Quebec Families

Sample Characteristics:

- 21 multigenerational families from Eastern Quebec, Canada
- Average of 6 affected individuals (SZ or BP) per family
- N = 480 family members (DNA available)
- 47 deceased affected ancestors included in pedigree analysis

Family Selection Criteria:

- At least one first-degree relative with same disorder as proband
- At least two additional affected relatives (first/second/third-degree)
- At least four affected members per family

Family Composition:

- 7 SZ pedigrees (85%+ affected by SZ spectrum among ill members)
- 6 BP pedigrees (85%+ affected by BP spectrum among ill members)
- 8 mixed pedigrees (both SZ and BP)

Part II: Methods Overview

Methods: The Big Picture

Overall Analytical Strategy:

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Step 1: Define phenotypes (who is "affected"?)
Step 2: Genotype families using microsatellite markers
Step 3: Perform model-based linkage analysis
Step 4: Calculate LOD scores across genome
Step 5: Apply significance thresholds (multiple testing)
Step 6: Identify candidate susceptibility regions
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Background Concept: What is Linkage?

Linkage = The tendency for genes/markers close together on a chromosome to be inherited together

- Linkage analysis requires pedigree data because it detects co-segregation of chromosomal regions through meioses

Key Principle (from Lecture 4):

- If a marker is **physically close** to a disease gene, they will **co-segregate** within families
- The closer they are, the less likely recombination occurs between them

Recombination Fraction (θ):

- θ = probability of recombination between two loci
- $\theta = 0.5$: loci are unlinked (different chromosomes or far apart)
- $\theta < 0.5$: loci are linked
- $\theta = 0$: loci are completely linked (no recombination)

Background Concept: Microsatellite Markers

What are microsatellites?

- Short tandem repeats (STRs) of 2-4 nucleotides (e.g., CA-CA-CA-CA, or CA-CA, etc.)
- Highly polymorphic: many different alleles in population
- Scattered throughout the genome

Why use microsatellites for linkage analysis?

- High heterozygosity $\rightarrow$ more **informative meioses**
- A pedigree contributes to LOD score only if key individuals are heterozygous
- More alleles = better ability to track inheritance

This study used:

- 607 microsatellite markers
- 350 markers at 10cM resolution (initial scan)
- 257 additional markers in promising regions

Methods Step 1: Phenotype Definitions

Three diagnostic classes analyzed:

Phenotype	Narrow Definition	Broad Definition
SZ	SZ only (N=71)	SZ+schizophreniform+ schizotypal (N=81)
BP	BP I only (N=48)	BP I+BP II+recurrent depression (N=72)
CL	SZ + BP I + schizoaffective (N=134)	CL narrow+depression +schizotypal (N=169)

CL = "Common Locus" phenotype

- Combines SZ and BP phenotypes
- Tests hypothesis that some loci are shared by both disorders

Diagnostic blindness: ensures that clinical diagnosis is assigned independently of family structure

Methods Step 2: Transmission Models

Model-based linkage analysis requires specifying:

1. **Mode of inheritance:** Dominant vs. Recessive
2. **Disease allele frequency:** How common is the risk allele?
3. **Penetrance:** P(affected | genotype)

Two models used in this study:

Parameter	Dominant Model	Recessive Model
Disease allele frequency	0.01	0.10
Penetrance (high age)	0.70	0.70
Phenocopy rate	0.20	0.10

Why test multiple models?

- True inheritance pattern is unknown for complex diseases
- Maximizing over models increases power but also Type I error

Background Concept: LOD Score

LOD (Log of Odds) Score measures evidence for linkage:

$$LOD(\theta) = \log_{10} \frac{L(\theta)}{L(\theta = 0.5)} = \log_{10} \frac{\text{Likelihood under linkage}}{\text{Likelihood under no linkage}}$$

When penetrance $f < 1$ or phenocopy rate $f_0 > 0$, the likelihood:

$$L(\theta) = \sum_{\text{genotypes}} P(\text{phenotypes}|\text{genotypes}, f, f_0) \cdot P(\text{genotypes}|\theta)$$

where for each individual i: $P(\text{phenotypes}|\text{genotypes}, f, f_0) = \prod_i P(Y_i|G_i)$

$$P(Y_i|G_i) = \begin{cases} f^{Y_i}(1-f)^{1-Y_i} & \text{if } G_i = \text{carrier} \\ f_0^{Y_i}(1-f_0)^{1-Y_i} & \text{if } G_i = \text{non-carrier} \end{cases}$$

Methods Step 3: Linkage Analysis Details

Two-point vs. Multipoint Analysis:

- **Two-point:** Tests linkage between disease and ONE marker
- **Three-point (multipoint):** Uses flanking markers together
  - More informative, extracts more information
  - Each marker included in two three-point analyses

Mod Score Approach:

- Maximize LOD score over θ AND over model parameters
- $$\text{Mod Score} = \max_{\theta,\text{models}} LOD(\theta)$$
- Tests 8 combinations: 2 phenotype levels (narrow *vs* broad) $\times$ 2 transmission models (dominant *vs* recessive) $\times$ 2 analysis types (affect/unaffected *vs* affected-only)

Software: FASTLINK (version of LINKAGE)

Methods Step 4: Multiple Testing Correction

The Problem:

- 607 markers $\times$ 3 phenotypes $\times$ 8 model combinations = many tests!
- High risk of false positives

Solution: Lander & Kruglyak (1995) Thresholds

Category	Z-score	Interpretation
Significant	≥ 4.0	Genomewide significant ($p < 0.05$)
Suggestive	≥ 2.6	Expected once per genome scan by chance
Confirmatory	≥ 1.9	Only in regions with prior evidence

Additional correction in this study:

- Raised thresholds by 0.70 (following Hodge et al.)
- Because: 24 non-independent analyses per marker

Methods Step 5: Heterogeneity Analysis

What is genetic heterogeneity?

- Same phenotype caused by different genes in different families
- Common in complex diseases

Problem: If only some families are linked to a locus, the overall LOD score may be diluted

Solution: Admixture Model (HOMOG program)

- Estimates proportion of families linked (α)
- Calculates HLOD (heterogeneity LOD)
- Applied to signals with mod score > 1.9

$$HLOD = \log_{10}\left[\alpha \cdot L(\theta) + (1-\alpha) \cdot L(\theta = 0.5)\right] - \log_{10} L(\theta = 0.5)$$

Part III: Results & Figures

Genetic Nomenclature: Loci and Markers

- **Chromosomal Location** (e.g., 15q11.1)
  - **15**: the chromosome number
  - **q**: the "long arm" of the chromosome (p stands for the "short arm")
  - **11.1**: the specific band and sub-band on that arm, numbered in increasing order as the distance from the centromere (the center) increases
- **Genetic Markers** (e.g., D15S122)
  - **D**: DNA
  - **15**: located on Chromosome 15
  - **S**: a single-copy sequence (a unique genomic locus)
  - **122**: the unique ID number for this specific marker
- **The Relationship**:
  - **15q11.1** defines a broad chromosomal region
  - **D15S122** is a precise genetic marker within that region, used to test linkage to a nearby disease locus

Results (see Table 1)

- **Significant Linkage Findings** (MOD Score $\geq$ 4.0):
  - **BP**: 15q11.1 (D15S122), 18q12.3 (D18S1145), 16p12.3 (D16S410)
  - **CL**: 15q26 (D15S1014), 18q21.1 (D18S472)
- **Suggestive Linkage Findings** (MOD Score $\geq$ 2.6):
  - **BP**: 12q23.1 (D12S1030), 3q21.2 (D3S3023), 10p13 (D10S674)
  - **SZ**: 6p22.3 (D6S334), 18q21.1 (D18S851), 13q13.3 (D13S1491)
  - **CL**: 16p13.1 (D16S3041), 2q22.1 (D2S298), 13q14.1 (D13S1247)
- **Novelty and Replication** (see Table 3):
  - **Novel Discovery**: the linkage signal at **15q26** for the shared phenotype
  - **Confirmatory Evidence** (MOD score $\geq$ 1.9): the finding at **3q21.2** for BP, **6p22.3** for SZ, **10p15-p11** for BP and SZ, **18q12.3-q32** for BP and SZ, etc.

Table 2: Family Contributions to the Genetic Signal

- The linkage signal is supported by several pedigrees rather than driven by a single influential family

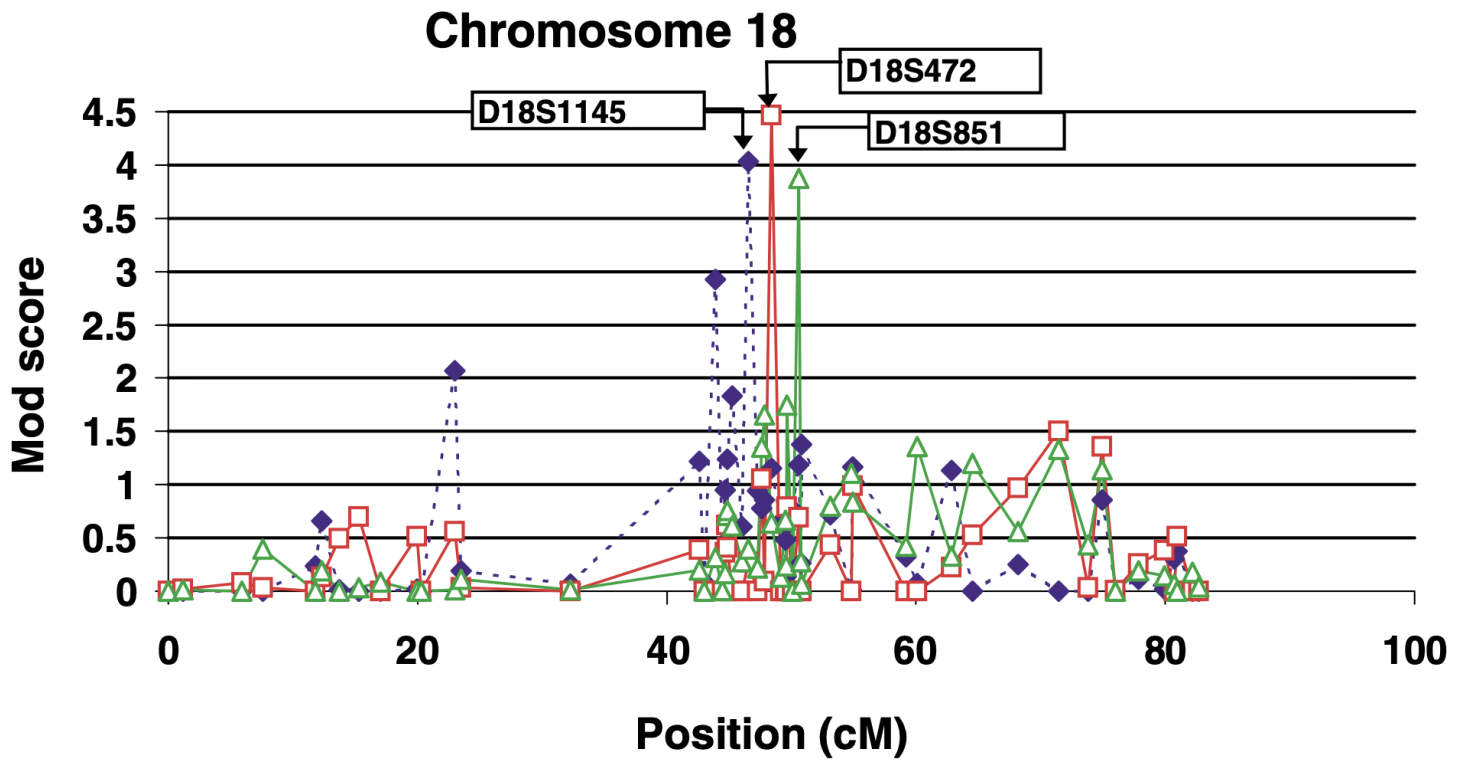
Table 2 Percentage of families according to their individual lod score contributing to the significant and suggestive total mod scores

Marker	*Mod score*	*Phenotype*	*Individual pedigree lod score interval*					*Highest family lod score*
			lod<0.1	*0.1≤lod<0.4*	*0.4≤lod<0.8*	*0.8≤lod<1.2*	*1.2≤lod*	
Genomewide significant linkage								
D15S122	4.59	BP broad	29%	21%	36%	14%	0%	1.04
D15S1014	4.55	CL narrow	29%	48%	19%	0%	5%	1.22
D16S410	4.05	BP narrow	21%	21%	57%	0%	0%	0.61
D16S3041	3.66	CL broad	33%	29%	38%	0%	0%	0.67
D18S1145	4.10	BP broad	43%	21%	21%	7%	7%	1.21
D18S472	4.46	CL narrow	33%	43%	24%	0%	0%	0.71
D18S851	3.87	SZ narrow	33%	53%	7%	0%	7%	2.03
Suggestive linkage								
D2S298	2.76	CL broad	52%	38%	5%	0%	5%	1.41
D3S3023	2.68	BP narrow	57%	14%	21%	7%	0%	1.04
D6S334	2.82	SZ narrow	40%	33%	7%	20%	0%	1.06
D10S674	2.66	BP narrow	50%	7%	43%	0%	0%	0.79
D12S1030	3.53	BP broad	21%	57%	14%	7%	0%	0.81
D13S1491	2.96	SZ narrow	33%	40%	13%	13%	0%	1.00
D13S1247	2.74	CL narrow	57%	19%	14%	5%	5%	1.69

For the analyses of the BP, the SZ and the CL phenotypes, the numbers of pedigrees under analysis were respectively 14, 15 and 21.

Figure 1: Visualizing Linkage across Chromosomes

- **X-axis**: physical distance along the chromosome (measured in cM)
- **Y-axis**: MOD score (the strength of the evidence)
- **Example**: Chromosome 18 shows overlapping linkage peaks for SZ, BP and the combined phenotype, suggesting a shared susceptibility locus rather than diagnosis-specific genes



Part IV: Discussion

Evidence for Shared Loci Between SZ and BP

- Data indicates overlapping susceptibility regions, suggesting that SZ and BP may share a common biological etiology in specific areas
- **Key Shared Hotspots**:
  - **Chromosome 18q**: showed consistent significant or suggestive linkage across SZ, BP, and combined phenotypes
  - **Chromosome 15q**: suggested the presence of one or more susceptibility genes contributing to either or both disorders
  - **Chromosome 16p**: primarily driven by BP and the shared phenotype, indicating a potential shared susceptibility locus; absence of a signal for SZ likely reflects diagnostic misclassification within pedigrees
- These results strongly support the hypothesis that some genetic risk factors are **not specific to one diagnosis** but are shared across the spectrum of these two major psychoses

Limitations

1. Statistical Thresholds

- Unclear if these criteria fit a small number of large families
- The effect of a two-step scan (sparse then dense) is hard to simulate

2. Sensitivity of Heterogeneity

- Current tests may fail to detect between-family heterogeneity

3. Complexity of Replication

- Difficult to define "true replication" across studies due to variations in:
  - Sampling & Diagnostics
  - Statistical methods & Genetic markers

Implications & Future Direction

- **Combination Model**: SZ and BP specificity arises from unique combinations of multiple shared genes
- **Modifier Gene Model**: SZ and BP share core genes, and specific modifier genes determine the final clinical phenotype
  - **Necessary but Not Sufficient**: Genes at loci like **6p22 (SZ)** or **16p12 (BP)** require interactions with other genes to trigger the disorder
  - **Affected-Only *vs* Affect/Unaffected**: More significant signals in AO analysis compared to AU
- **Future Directions**
  - The presence of multiple signals in one sample may allow for the study of gene-gene interactions (epistases)
  - Expand statistical power using a second sample of 500 members, ultimately identifying defective genes and paving the way for new treatments

Questions for Class Discussion

1. Why did the authors use **model-based** (parametric) linkage analysis instead of model-free methods for this study? What are the trade-offs?
2. The authors used both **narrow and broad** phenotype definitions. How might the phenotype definitions affect LOD scores and power?
3. We learned that LOD scores require specifying **penetrance** values. This study used age-dependent penetrance. Why is this important for late-onset disorders?
4. The maximum family LOD score was only ~2.0 (Table 2), yet the combined scores reached significance. What does this tell us about **genetic heterogeneity** of these disorders?
5. This is a **two-stage design** (initial sparse scan + dense follow-up). What are the advantages of this approach compared to performing dense genotyping from the start?